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# A HISTOCHEMICAL STUDY OF OOGENESIS IN THE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS*[1]

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The sea urchin has an annual reproductive cycle with specific important cellular events occurring at certain times of the year (Caullery, 1925; Fuji, 1960; Holland, 1967; Holland and Giese, 1965; Moore, 1936; Pearse, 1969a, 1969b; Pearse and Giese, 1966; Pearse and Phillips, 1968; Tennent and Ito, 1941). These stages are clearly outlined by Fuji (1960) who split the yearly cycle into five stages: (I) recovering spent, characterized by a few primary oocytes along the wall of the ovary; (II) growing, larger oocytes line the walls; (III) premature, large oocytes along the walls and a few mature ova in the central lumen; (IV) mature, fertile with mature ova; and (V) spent. Not only gametes but also the accessory cells which Holland and Giese (1965) refer to as nutritive phagocytes are found to undergo seasonal changes (Dawydoff, 1948; Holland and Giese, 1965).

Other studies on sea urchin oogenesis deal with the dictyotic stage of Holland and Giese (1965) or what Tennent and Ito (1941) refer to as the diffusion and resting nucleus stage of the primary oocyte when the ovary is in the mature stage of Fuji (1960). These studies are concerned with the synthesis of RNA, protein, and polysaccharides in the nucleolus, and the transfer of these materials to the cytoplasm (Cowden, 1962; Esper, 1965; Ficq, 1962, 1964; Ficq, Aiello and Scarono, 1963; Gross, Malkin and Hubbard, 1965; Piatogorsky, Ozaki, and Tyler, 1967).

Recently, electron microscope studies have been made on sea urchin oogenesis that concentrate mainly on morphological changes of the sex cells and do not deal with seasonal variations (Anderson, 1968; Verhey and Moyer, 1967a).

The present study was undertaken to correlate the morphological and biochemical changes in the egg cells and nutritive phagocytes with relation to the annual reproductive cycle, by histological and histochemical techniques.

## Material and Methods

Specimens of *Strongylocentrotus purpuratus* were collected at two week intervals from October 1966 to March 1967 in the tide pools of the mean low tide zone of Yaquina Head, Oregon (latitude 44° 40′ 40″, longitude 124° 4′ 40″). This is the period in which the greatest proliferation of the gonad and the spawning of the eggs takes place. In addition samples of spent ovaries were sampled in April and May of 1966. Although no animals were obtained from June through September,

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the May and October specimens overlapped sufficiently to present a continuous spectrum.

The sea urchins were sacrified on the same day as collected or the morning immediately following a night collection. The wet weights of the animals were taken; the urchins were then injected with 2 cc of 0.5 *M* KCl, and allowed to stand for half an hour to see if they would spawn. Gonads that could not be sexed by macroscopic inspection and all ovaries were removed from the urchins and fixed in Bouin's, Carnoy's or calcium formal fixatives. The fixed material was then imbedded in gelatin for frozen sectioning or in paraffin. The gelatin imbedded tissues were quick frozen and sectioned on a microtome in a Model CTD International Harris Cryostat. Ten micron sections were made of both frozen and paraffin sections.

The hematoxylin and eosin method was used for standard histological examination. Oil red O counterstained with Mayer's hematoxylin was used for neutral lipids; Feulgen reaction, for deoxyribonucleic acid (DNA); methyl green, pyronin (MGP) method was used for ribonucleic acid (RNA) in combination with mild acid hydrolysis of parallel sections, 1 *N* HCl at 60° C for five minutes, as a control. The periodic acid Schiff (PAS) technique was used for the demonstration of polysaccharides with diastase digestion of parallel sections to indicate the presence of glycogen in the tissue. All histochemical techniques used followed the schedules as outlined in Barka and Anderson (1963). In addition, the presence of RNA was determined by the azure B technique of Flax and Himes (1952) as modified by Szollosi (1965). Mild acid hydrolysis of parallel sections was again used to verify the presence of RNA.

Photomicrographs were made on a Leitz Wetzlar microscope with an Olympia 35 mm camera.

## Results

The sea urchin has five separate ovaries; each covered by a flagellated peritoneal epithelium. The ovaries are large rebranched sacs and each saccule ends in a blind acinus. The wall of the ovary under the peritoneum is made up of collagenous connective tissue and smooth muscle. In the central portion of each acinus are two main cell types: the sex cells, which mature into ova, and the accessory cells, also referred to as nutritive phagocytes.

In discussing the yearly reproductive cycle of *Strongylocentrotus purpuratus* the five stages of Fuji (1960) are used. The yearly gross and histological changes are summarized in Table I and temperature and salinity data, in Table II.

### *Recovering spent stage*

This stage occurs, in urchins found along the central Oregon coast, from the late summer months to early fall. There is, however, a great deal of individual variation in the timing of this stage and also in the timing of the other stages. The ovary in the recovering spent stage is small but firm and is slightly larger and more substantial than ovaries that have just been spent. Its color varies from tan to orange depending on the number of dark brown degenerating bodies (discussed below) in the ovary and the amount and size of the immature eggs.

TABLE I

*Ovarian cycle*

| Months most common | Recovering spent I | Growing II | Premature III | Mature IV | Spent V |
|---|---|---|---|---|---|
| | Aug.–Oct. | Oct.–Nov. | Nov.–Jan. | Jan.–Apr. | Apr.–Aug. |
| Oogonia | very few | few | more numerous | numerous | very numerous |
| Dictyotene oocytes | 10–20 μ | 20–30 μ | up to 60 μ, all sizes | same | none growing 5–10 μ |
| Ova | none | none | few | many | degenerating |
| Nutritive phagocytes | lightly globulated | heavily globulated | same | emptying | empty and refilling |
| External appearance | dark brown firm, small | brown to orange, firm medium | brown to orange "fluffy" full size | orange, ripe full size | dark brown flaccid, small |

In histological section the nutritive phagocytes completely fill the entire ovary, obscuring the lumina except for the small oocytes that abut against the wall of the ovary. These nutritive phagocytes contain eosinophilic droplets (Fig. 1), but the droplets are not as densely packed nor as numerous as in later stages. The globules stain very intensely with the periodic acid Schiff method, indicating large amounts of polysaccharides (Figs. 3 and 4). Glycogen extraction only reduces the intensity of the stain evenly over the entire ovary, indicating that while glycogen may be present in large amounts, other polysaccharides may make up the greater percentage of these globules or the glycogen is some how protected from the action of the enzyme. These cells also contain many lipid inclusions (Fig. 2), and their nuclei are Feulgen positive.

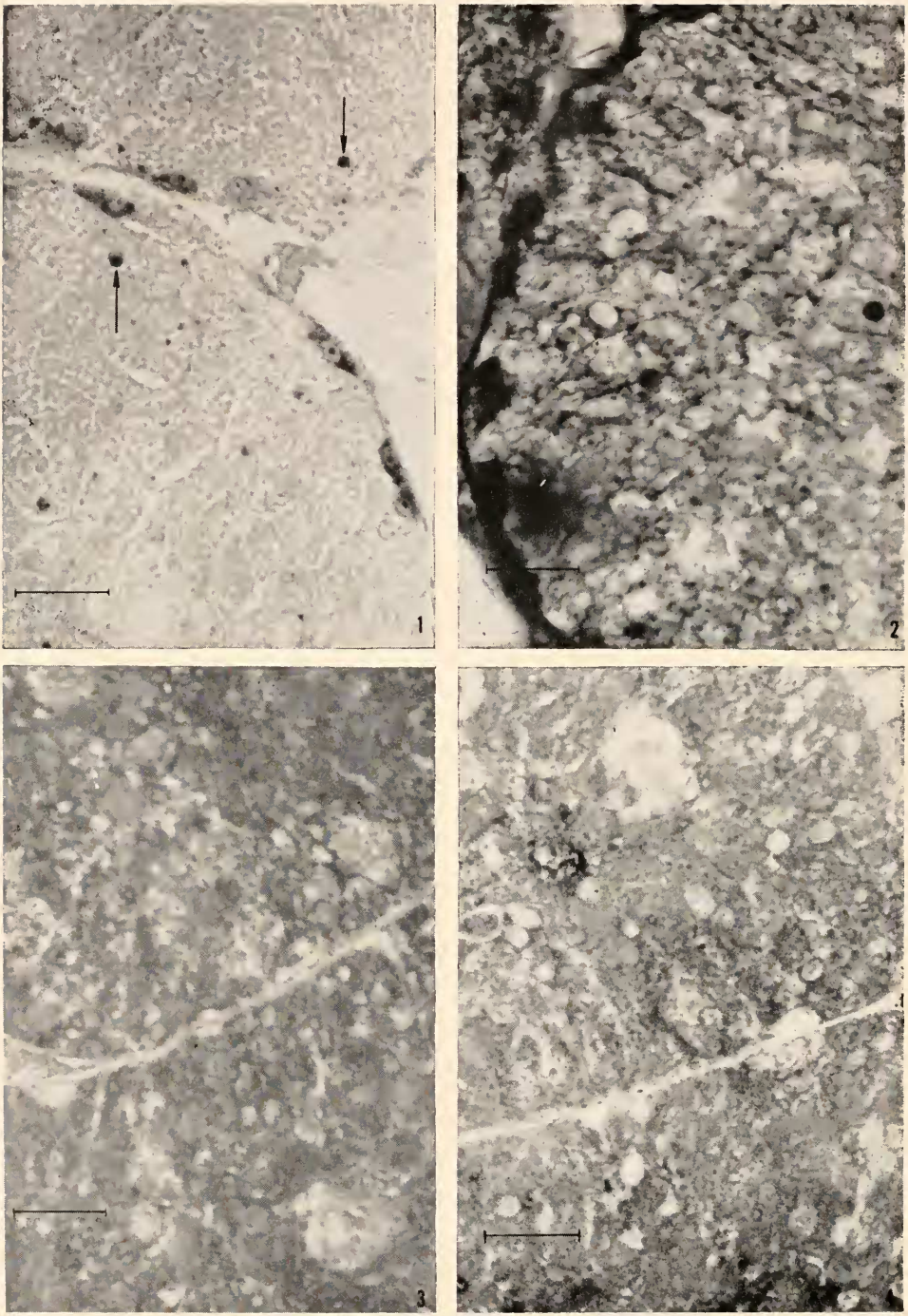
Among the nutritive phagocytes many sex cells can be seen with an irregular or indistinct outline. Also, dark brown granules (Figs. 1 and 5) and numerous basophilic inclusions (Fig. 6) are present, although the granules and inclusions are somewhat more common in later stages. The brown granules are Feulgen positive, indicating they contain DNA and are probably breakdown products of nutritive

TABLE II

*Temperature and salinity ranges**

| | Aug.–Oct. | Oct.–Nov. | Nov.–Jan. | Jan.–Apr. | Apr.–Aug. |
|---|---|---|---|---|---|
| Surf temperature °C Newport 1966 | 10–15 | 10–14 | 9–12 | 8.5–11 | 8–16 |
| Surf temperature °C Depoe Bay 1967 | 13–18 | 10–13 | 10–12 | 9–11 | 11–18 |
| Salinity ‰ Newport 1966 | 32–34 | 29–33 | 22–32 | 27–33 | 28–33 |
| Salinity ‰ Depoe Bay 1967 | 28–34 | 32–34 | 32–34 | 29–32 | 28–34 |

* Temperature and salinity data from Wyatt and Gilbert, 1967 and Gilbert and Wyatt, 1968.




FIGURES 1–4.

phagocytes, developing eggs or perhaps both (Fig. 7). There are often other basophilic granules in or between the nutritive phagocytes that contain concentrations of RNA and occasionally others which contain both DNA and RNA.

Oogonia are very difficult to find in the recovering spent ovary; they occur singly or in very small groups scattered along the wall of the ovary. These oogonia, whose diameter is an average of five microns, have a scanty cytoplasm that does not stain specifically. Generally, two small dense nuceoli distinguish them from primary oocytes of the same size. The nuclei of the oogonia are slightly Feulgen positive.

Many small primary oocytes just beginning their growth or dictyotic phase, resting or diffusion nucleus stage of Tennent and Ito (1941), can be seen along the wall of the ovary (Fig. 1). There is generally an interval of about 15 microns between each one and they are slightly elongated in their axis parallel to the ovarian wall with their narrowest width ranging from 10–20 microns. The cytoplasm of these small oocytes shows a great concentration of azure B positive material which does not stain with pyronin (Fig. 6). Although some polysaccharide is present in the cytoplasm of the oocytes at this stage, it is very scant compared with the dense concentration in the adjacent phagocytes. No lipid appears in the oocytes at this time and no Feulgen stain can be seen in the germinal vesicle.

The most prominent feature of the young oocytes is the nucleolus, which is three to five microns in size. At this stage it often appears to be more eosinophilic than the cytoplasm, for it contains not only material stainable with both pyronin (Fig. 8), and azure B (Figs. 6 and 14), but also diastase extractable and non-diastase extractable polysaccharides. Despite this the nucleolus appears very dense and homogeneous during this stage.

*Growing stage*

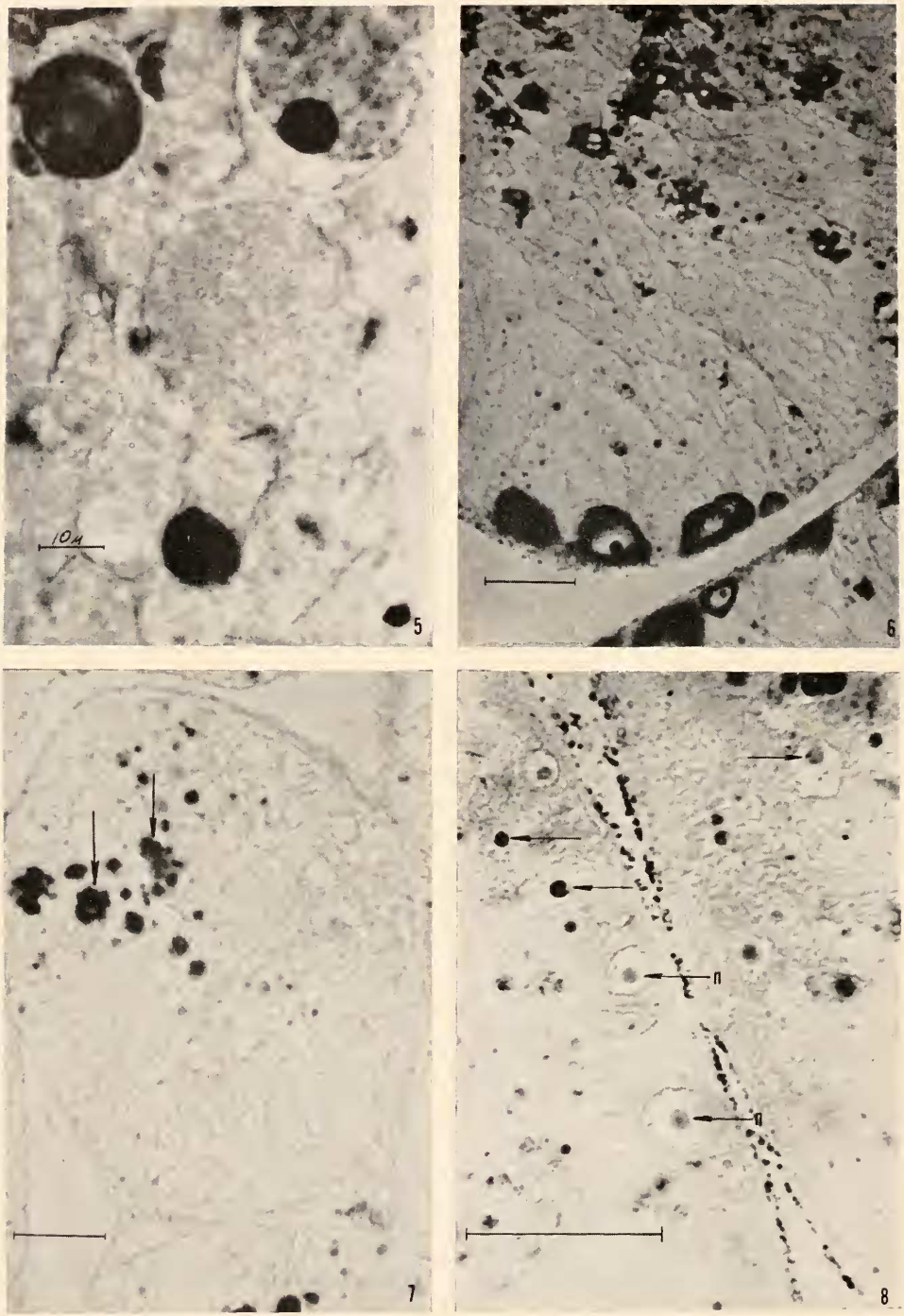
In the late fall the ovaries enter the growing stage during which time they greatly increase in size. (See gonad index for *S. purpuratus* at Coos Bay, Boolootian, 1966.) They have a mealy texture and vary in color from a tan to the golden orange color of mature eggs. At the cellular level, the nutritive phagocytes are densely filled with polysaccharide and lipid globules (Figs. 9, 10, 11, and 12), and large vacuoles are present in many of these cells (Fig. 12). The phagocytes have attained their maximum size of 15–20 microns in diameter, and are irregular in shape due to crowding. Their cytoplasm is pyroninophilic at this stage (Fig. 13) but only a barely perceptible bluing occurs with azure B. Many oocytes seem to have indistinct borders where they abut with phagocytes, although this is difficult to ascertain exactly with the light microscope (Figs. 14 and 15).

FIGURE 1. Acinus of late recovering spent ovary. Note small primary oocytes along walls. Accessory cells (nutritive phagocytes) are filled with eosinophilic globules and have large dark granular inclusions (arrows). Hematoxylin and eosin (H&E). Micron markers on micrographs represent 50 microns unless otherwise indicated.

FIGURE 2. Late recovering spent ovary. Nutritive phagocytes take up lipid stain. Small oocytes along ovarian wall appear very dark and stain only with hematoxylin. Gelatin imbedded; oil red O and Mayer's hematoxylin (H&O).

FIGURE 3. Late recovering spent ovary. Accessory cells full of polysaccharides. Periodic Acid Schiff (PAS).

FIGURE 4. Parallel section to one in Figure 3 with glycogen extracted with diastase. PAS.




FIGURES 5–8.

The oocytes have attained a size of 20–30 microns in diameter; most are slightly elongated in the axis perpendicular to the acinar wall, while some are spherical. The cytoplasm of these oocytes is similar to that described in the previous stage, but the azure B stain is not as intense.

On the other hand, a slight change has occurred in the nucleolus; it is more intensely pyroninophilic than is the previous stage and often possesses one or two small vacuoles (Fig. 14). It has not kept pace with the growth of the rest of the cell and appears to be the same size as it was in the previous stage. Scattered particles appear in the germinal vesicle that stain with hematoxylin in the same way that the nucleolus does, but these particles do not stain with azure B, pyronin, or PAS.

*Premature stage*

In this stage, which occurs in the early winter, the ovaries are golden orange and take on the outward appearance of mature ovaries. Although there are a few ripe eggs present in the ovary, they will not be shed even with the stimulus of KCl injections. The nutritive phagocytes remain much as described in the previous stage, but those in the more central portions of the ovary have lost their pyroninophilia. Nests of oogonia and primary oocytes in the pre-dictyotic phases are now quite frequent (Fig. 16).

The uniformity in size of the growing oocytes is lost, for all sizes can be found from the smallest to the largest which are about 50–60 microns in diameter, the size of the mature ovum. The smaller oocytes are usually found in the peripheral acini. The cytoplasm of these small oocytes stains much more intensely with azure B than that of larger oocytes (Fig. 17), while the larger oocytes contain much more polysaccharide (Fig. 18), and lipid appears in their cytoplasm for the first time (Fig. 19). Although these two constituents are also found in the globules of nutritive phagocytes, they occur in the cytoplasm of the oocyte as much finer grains. The nucleolar vacuoles become larger and more numerous and make up a large portion of the nucleolar area in the biggest oocytes. Usually these vacuolated nucleoli have lost their pyroninophilia, but still show visible staining with the azure B and the PAS techniques.

At the time of the maturation divisions the germinal vesicle becomes ill-defined as its membrane breaks down and the nucleolus disappears. The oocyte either moves or is pushed toward the center of the acinus where meiosis takes place. This movement displaces the nutritive phagocytes that had formerly been in the center, but no patent lumen is formed. The spindle of the maturation divisions forms near the plasma membrane on one side of the egg, thus producing small polar bodies.

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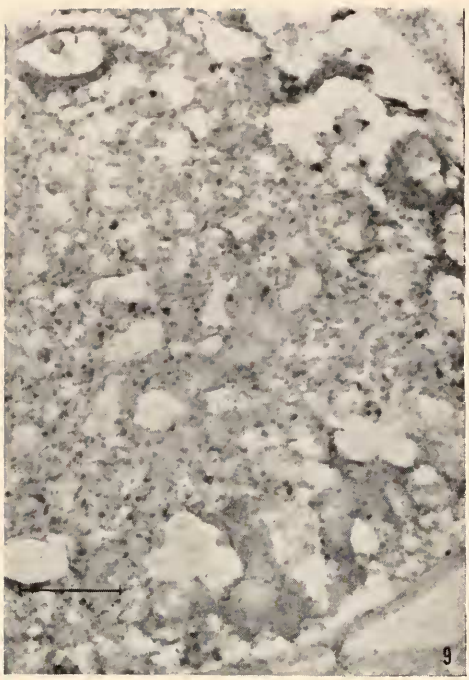
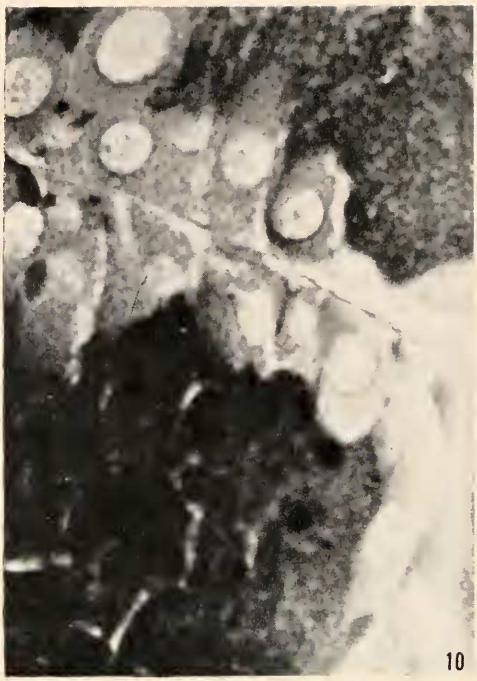
FIGURE 5. Late recovering spent ovary. Inclusions of nutritive phagocytes. Note large dark granular inclusions and inclusion that looks like the germinal vesicle of an oocyte with its nucleolus in upper right corner. H&E.

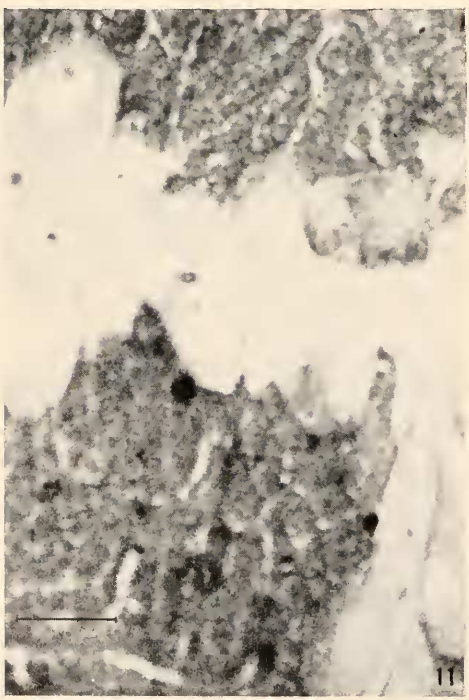
FIGURE 6. Late recovering spent ovary. Cytoplasm of small oocytes stain dark blue indicating the presence of RNA. Basophilic inclusions of nutritive phagocytes (top of picture) stain purple. Azure B.

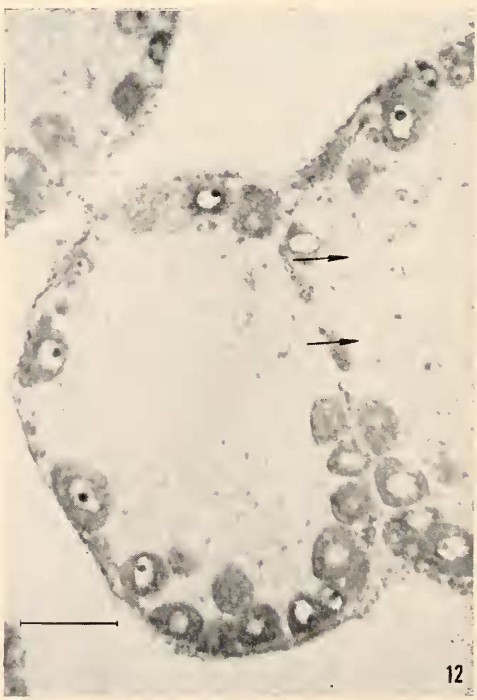
FIGURE 7. Mature ovary. Large dark granular inclusions (arrows) are Feulgen positive. Feulgen.

FIGURE 8. Growing ovary. Nucleoli (n) of germinal vesicle are pyroninophilic. Large dark granular inclusions stain with methyl green (arrows). Methyl green and pyronin (MGP).




FIGURES 9–12.

As the season progresses the ovary becomes studded with the small pyknotic nuclei of the polar bodies, which can be identified most readily with the Feulgen stain. Few eggs can be found undergoing maturation divisions at any one time and it is often necessary to hunt carefully in serial sections to find any at all. The nucleus of the mature ovum is very small, about five microns in diameter, as compared to the germinal vesicle of the oocytes which is 20–25 microns in diameter. The chromosomes, which are now concentrated enough to be visibly stained by the Feulgen method, can be seen as small droplet-like areas adhering to the nuclear membrane.

*Mature stage*

The ovaries usually become mature about midwinter and the breeding season commonly lasts well into the spring. The ovaries are colored a golden orange by the mass of mature ova which they contain. They will shed after almost any mild shock and with KCl they will shed almost immediately.

There is often a large pyroninophilic area in the ova that are about to be shed and those that have already been shed. While still within the ovary this area is irregularly shaped, as are the ova themselves due to crowding, but once the ova are released both they and the pyranophilic area within them assume their normal spherical shape (Fig. 20).

The mature ova within the ovary have displaced the nutritive phagocytes to the outside of the acinus where they form a single layer containing oocytes that have not yet matured (Fig. 21). The more peripheral acini retain an aspect similar to that found in the premature ovaries, except that there tends to be a great variation in the sizes of the oocytes (Fig. 22). Usually the smallest oocytes, ten microns in diameter, occur in clusters in association with one or more nests of oogonia and pre-dictyotic oocytes, all of which become more frequent as the season progresses.

Due to the presence of continually growing oocytes the urchin is able to shed several times during the breeding season. A definite, progressive change can be noted in the phagocytes over this time. The change starts with the cells in the more central portion of the ovary and proceeds toward the more peripheral acini over the course of the season. The lipid and polysaccharide droplets within these cells become fewer in number (Fig. 23) and eventually are entirely depleted in the spent stage (Fig. 24). At this point some of the phagocytes disappear, but many remain, identifiable only by their plasma membrane, small nucleus, and a few dark granular inclusions.

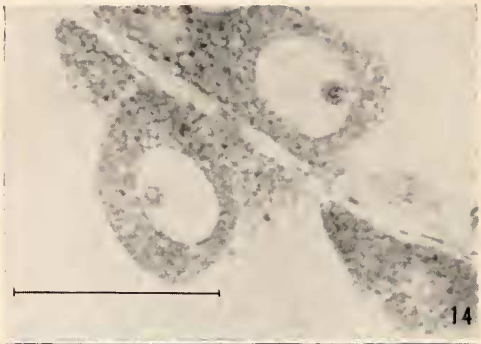
Toward the end of the breeding season the size of the oocytes becomes progressively smaller as less cytoplasm is produced. They are elongated as opposed

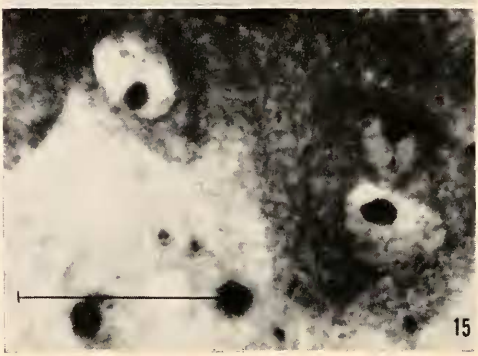
FIGURE 9. Growing ovary. Nutritive phagocytes stain with oil red O and oocytes stain with hematoxylin. H&O.

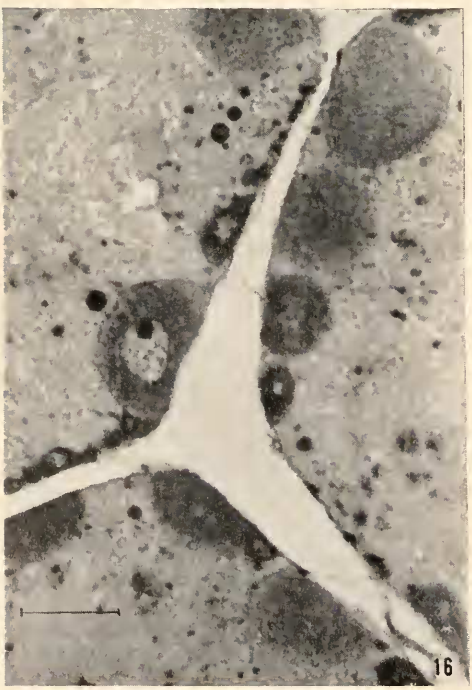
FIGURE 10. Growing ovary. Oocytes do not stain as darkly as the surrounding accessory cells. Note nucleolus of germinal vesicle is PAS positive. PAS.

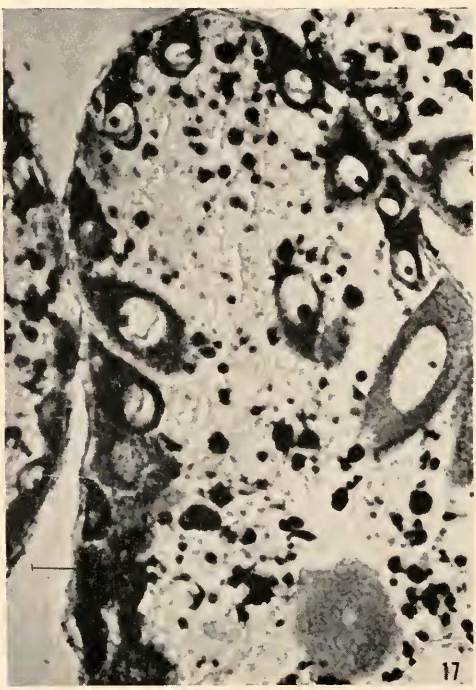
FIGURE 11. Parallel section to one in Figure 10 with glycogen extracted with diastase. PAS.

FIGURE 12. Growing ovary. Individual eosinophilic globules can no longer be distinguished as they can in Figure 1. Note vacuoles in nutritive phagocytes (arrows). H&E.


FIGURES 13–17.

to the spherical or elliptical shape common earlier in the season (Fig. 24). Usually these oocytes are not shed even if they do mature and the ovary is now termed spent.

*Spent stage*

At this time the external aspect of the ovary looks very much reduced, flabby, and has a dark brown color due to the presence of brown degenerating bodies. The nutritive phagocytes are empty, giving the inside of the ovary an open mesh appearance. Quite a few deteriorating, unshed ova can be seen in the now open lumina (Fig. 25).

One very interesting and important event occurs at this time: the oogonia, pre-dictyotene oocytes, and very small dictyotene oocytes that have been slowly increasing in numbers over the course of the year are now quite numerous (Fig. 25). Together these various types of germ cells form a layer of two or three cells thick just under the wall of the acini. As fall approaches the number oogonia and oocytes decreases, but some of the small oocytes remain and begin growing along the wall of the ovary (Fig. 1). The nutritive phagocytes begin refilling with globules as the ovary once again enters the recovering spent stage.

## Discussion

A study of the annual reproductive cycle of the sea urchin shows that there is no period of the year when the ovary can be considered dormant. The cycle can roughly be divided into two parts: the first when the phagocytes are filling with globules and the majority of sex cells are in the dictyotene phase, and the second when the phagocytes are being depleted of their globules and sex cells in stages earlier than the dictyotene phase are numerous. Since the large growing oocytes are most plentiful at that time of year when the phagocytes are full of globules it seems that the globules are necessary for oocyte growth, especially since polysaccharide and lipid appear in the phagocytes before they are seen in the cytoplasm of the oocytes.

Although indistinct borders do occur between phagocytes and growing oocytes as seen with the light microscope (Figs. 14 and 15), it is unclear whether this represents an engulfment of the oocyte by the phagocyte or active transport in the reverse direction. While Miller and Smith (1931) report incorporation of degenerating accessory cells by the oocytes in *Echinometra lucunter,* the present author concurs with Holland and Giese (1965) in that no phagocytosis of the nutrive cells by the oocytes was observed in *S. purpuratus.* Takishima and Takishima (1965) report that glycogen particles in the ovaries of *Hemicentrotus pul-*

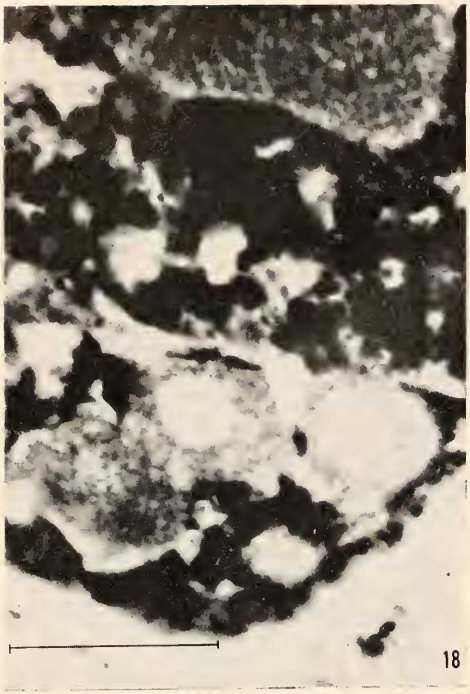
Figure 13. Growing ovary. Nutritive phagocytes have pyroninophilic areas (arrows). Dark granular inclusions are stained with methyl green. MGP.

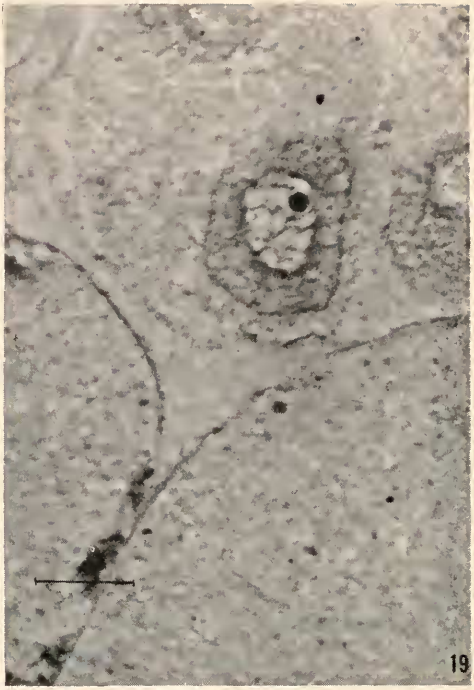
Figure 14. Growing ovary. Primary oocytes in dictyotic stage. Nucleoli contain vacuoles. Note border of the upper large oocyte is indistinct. Azure B.

Figure 15. Growing ovary. Primary oocytes with indistinct boarders. H&E.

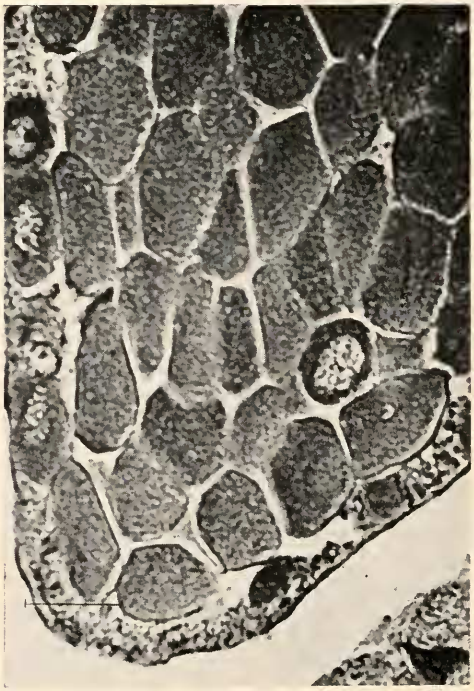
Figure 16. Premature ovary. Note variety in size of egg cells lining the wall of the acinus. H&E.

Figure 17. Premature ovary. Smaller oocytes stain more intensely with stain for RNA than larger ones and ova. Basophilic inclusions in nutritive phagocytes similar to those in Figure 6. Azure B.







FIGURES 18–21.

*cherrimus* are released by the accessory cells into an intercellular space and that these particles are in turn taken up by the oocyte by pinocytosis. However, Verhey and Moyer (1967a) report that no pinocytotic vesicles occur in the plasma membrane of the oocytes of *Arbacia punctulata, Lytechinus variegatus,* and *L. pictus* and concluded that RNA and proteins elaborated in the nutritive phagocytes are not taken up by the oocytes; however these authors did not study the oocytes over the course of the year, and it may be that pinocytosis occurs at a stage during the annual reproductive cycle other than the one they investigated. The same authors (Verhey and Moyer, 1967b) also found none of the polysaccharide in the accessory cells of the sea urchins they examined was glycogen. On the other hand *S. purpuratus* has large amounts of glycogen in its accessory cells as well as polysaccharide that is not extracted with diastase (Figs. 3, 4, 10, 11 and 18). Even this non-extracted polysaccharide may be glycogen that is somehow protected from the action of the diastase.

While it is not clear if the sex cells obtain nutriment directly from the phagocytes, there is evidence, on the other hand, that the phagocytes engulf sex cells. Fuji (1960) reports absorption of unshed degenerating ova, and Pearse (1969b) reports breakdown and subsequent phagocytosis of smaller oocytes when larger ones nearing maturity are numerous. Many phagocytes in the recovering spent phase contain RNA and DNA positive inclusions that under close inspection with the light microscope appear to be degenerate nuclei of early dictyotene oocytes (Fig. 5). Certainly this mechanism would account for the drastic reduction from the large number of immature sex cells seen along the walls of the spent ovary to the amount seen in the early growing stage. This raises the question of whether the phagocytes exercise some control on the number of oocytes that can develop. Alternatively, Holland (1967) suggests that growing oocytes might have an inhibitory effect on smaller primary oocytes.

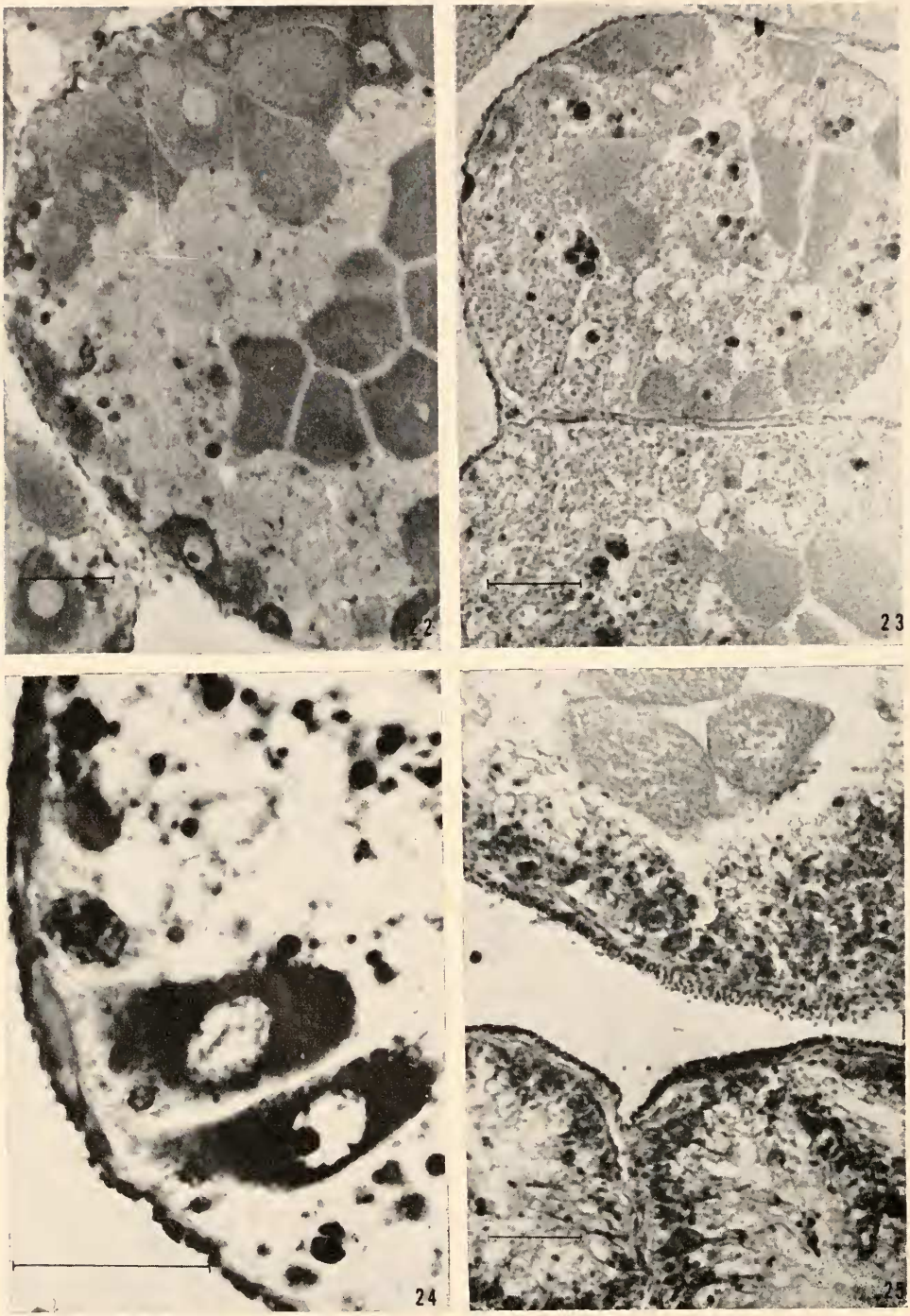
The cyclical appearance and disappearance of the globules within the phagocytes poses another important question. If the oocytes do indeed derive nutriment from the phagocytes, it is easy enough to explain their deglobulation. However, it is unlikely that the phagocytes are completely refilled by incorporating degenerating ova or developing oocytes for there are not enough of these to account for the abundance of polysaccharide and lipid that appears within them during the globulated stage. It is more reasonable to suppose that this variation is due to greater availability of food in the animals' habitat during the time when the phagocytes are filling and its relative scarcity when they are becoming depleted. Fluctuation in the food supply may likewise explain the great variability in gonad size and fertility from year to year (Boolootian, 1966). This however, is probably not the full answer, for Japanese urchins show a decline in food consumption during the premature and mature stages which does not correlate with a decline in

FIGURE 18. Mature ovary, peripheral acinus. Oocytes do not stain as intensely as ova. PAS.

FIGURE 19. Premature ovary. Oocytes appear purple because they stain with both lipid stain and hematoxylin. Nucleolus of germinal vesicle stains only with hematoxylin. H&O.

FIGURE 20. Mature ovary. Pyroninophilic area occurs in ova. MGP.

FIGURE 21. Mature ovary, central acinus. The numerous ova "push" nutritive phagocytes and growing oocytes into a single layer against the wall of the acinus. Ova are irregular in shape due to crowding. H&E.




FIGURES 22–25.

food supply (Fuji, 1967). Holland (1967), studying *Stylocidaris affinis*, thought photoperiod might be used as a reference point to synchronize an endogenous reproductive rhythm.

It has also been suggested (Giese, 1959) that temperature and salinity may have some influence on the timing of the reproductive cycle. From the data available (see Tables I and II) there seems to be a correlation between the filling of the phagocytes and temperature, but none with salinity. The water temperatures and salinities recorded are those of the open surf and would be correct for subtidal urchins; however, the intertidal ones caught in the tide pools would probably be exposed to much higher temperatures and increased salinity due to evaporation.

The seasonal variation in the amount of oogonia and pre-dictyotene oocytes raises the question of where the additional oogonia come from. Tennent and Ito (1941) suggested that they were derived from the peritoneal epithelium, but they were unable to present any proof. Although the incorporation of DNA precursors was followed over the course of one year (Holland and Giese, 1965), this study did not give any support to Tennent and Ito's theory and in fact indicates that oogonia are derived from previously existing ones by mitosis. The cells of the visceral epithelium are highly differentiated flagellated cells of the type described by Lyons, Bishop, and Bacon (in preparation) and occur commonly in other parts of the visceral epithelium as well. It seems unlikely that these cells would redifferentiate into germ cells.

No positive Feulgen reaction is seen in the germinal vesicles of the dictyotene oocytes because the DNA is presumably too dilute to be detected by this method. In the mature egg a Feulgen reaction can again be seen for the chromosomes have recondensed as small droplet-like areas adhering to the nuclear membrane. This configuration is apparently characteristic of sea urchins for it has also been reported by Burgos (1955) in *Arbacia punctulata* and by Agrell (1958, 1959) in *Paracentrotus lividus, Arbacia lixula,* and *Spantangas parcus.*

The discrepancies between the results obtained with the pyronin and azure B methods for staining RNA can be explained if it is possible that they are staining different species of RNA. It must be remembered that the basis of the behavior of these two staining techniques has not been definitely worked out, and therefore the following discussion is conjectural. However, from what is presently known about these two stains it is possible to assume they act somewhat differently. Immers (1961) stained unfertilized sea urchin eggs with pyronin and stated that if the phosphate groups of RNA are combined with protein as they are when the RNA is actively synthetic, the RNA will not take up the stain. On the other hand Flax and Himes (1952) state that azure B competes with protein for the phosphate groups of RNA and therefore is apparently able to stain RNA that would normally be combined with protein by replacing the protein. If this is true, then the azure B stains RNA that is more highly saturated with protein and there-

FIGURE 22. Mature ovary, peripheral acinus. Note variety in size of sex cells. H&E.

FIGURE 23. Mature ovary, toward end of breeding season. Individual eosinophilic globules can once again be seen in nutritive phagocytes. H&E.

FIGURE 24. Spent ovary. Large oocytes are elongated. No eosinophilic globules occur in nutritive phagocytes, only dark granular inclusions. Many very small primary oocytes and oogonia occur along acinus wall. H&E.

FIGURE 25. Spent ovary. Degenerating ova in open lumen. Great proliferation of very small primary oocytes and oogonia along wall of acinus. H&E.

fore presumably synthetically active while pyronin stains the relatively inactive RNA that is not highly saturated with protein. On this basis the growing oocytes contain actively synthetic RNA, while the RNA found in the nutritive phagocytes is mainly inactive. Alternatively, this staining phenomenon may have something to do with the fact that some of the RNA is blocked by a protein inhibitor while the rest is not. The appearance of pyroninophilia in mature ova (Fig. 20) may correspond to the inactive form of messenger RNA said to be present in unfertilized sea urchin eggs (see reviews by Grant, 1965; Gross, 1967; Spirin, 1966). The position and time of appearance of the pyroninophilia may correspond to the appearance of the heavy bodies (Afzelius, 1957; Harris, 1967) for it rarely occurs in the oocytes before they have undergone the maturation divisions and likewise heavy bodies do not appear until this time (Verhey and Moyer, 1967a. The captions of Figs. 19 and 20 of their report refer to ova as mature oocytes which makes interpretation of their electron micrographs rather confusing).

Chaet (1966) and Scheutz and Biggers (1967) have shown that there is a hormone that can be extracted from the radial nerve of the starfish, *Asterias forbesi,* that induces germinal vesicle breakdown and subsequent maturation division. It is possible that a similar mechanism may be at work in sea urchins and it may also cause the oocytes to migrate from the ovarian wall to the central lumen. Another possibility is that the larger oocytes may be capable of limited ameoboid movement that is largely suppressed by the crowding of the nutritive phagocytes. When the phagocytes are smaller and deglobulated at the end of the breeding season, they no longer suppress this ameoboid movement and the oocytes take on a very elongated shape (Fig. 24).

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## Summary

1. Oogenesis in the sea urchin *Strongylocentrotus purpuratus* was studied by histological methods and by histochemical techniques for polysaccharides, lipids, and nucleic acids. Urchins were collected at Yaquina Head, Oregon at regular intervals between April 1966 and March 1967. An attempt was made to correlate seasonal variations in coastal water temperature with the gonadal cycle.

2. Oogonia can be found throughout the year in small groups scattered along the walls of the ovary, but are most numerous in the late spring and early summer when the ovary is spent. The oocytes start growing in the late summer and early fall when the accessory cells start filling with lipid and polysaccharide globules. At this time the accessory cells are found to have inclusions that appear to be degenerate sex cells. The oocytes continue to grow through the late fall and early winter and their cytoplasm fills with lipid and polysaccharide. As the ova mature they move from the wall to the central portion of the acinus where they displace the accessory cells that had formerly been there.

3. The ova that have been shed or are about to be shed contain pyroninophilic RNA which is not found in the cytoplasm of the oocytes. However, both ova and

oocytes have RNA that is stainable with azure B. The pyroninophilic RNA is also found in accessory cells.

4. Since all the oocytes do not mature at the same time, a sea urchin is able to shed many times during the breeding season which lasts from late December to early April. During this period the accessory cells progressively lose their globules. When the accessory cells are finally depleted of their lipid and polysaccharide, the oocytes no longer grow and the ovaries are spent.

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